Title: Type 2 Diabetes and Metformin Use Associate With Outcomes of Patients With Non-alcoholic Steatohepatitis-related, Child-Pugh A Cirrhosis

Short title: Type 2 diabetes and outcomes in NASH cirrhosis

Eduardo Vilar-Gomez[1*], Luis Calzadilla-Bertot[2*], Vincent Wai-Sun Wong[3], Marlen Castellanos[4], Rocio Aller-de la Fuente[5], Mohammed Eslam[6], Grace Lai-Hung Wong[3], Jacob George[6], Manuel Romero-Gomez[7], Leon A. Adams[2,8].

[1] Division of Gastroenterology and Hepatology, Indiana University School of Medicine, Indianapolis, Indiana, USA.

[2] Medical School, Faculty of Health and Medical Sciences, The University of Western Australia, Nedlands, Australia.

[3] Institute of Digestive Disease, The Chinese University of Hong Kong, Hong Kong, China.

[4] Department of Hepatology, National Institute of Gastroenterology, Havana, Cuba.

[5] Department of Digestive Disease, Clínico Universitary Hospital, Valladolid. Institute of Endocrinology and Nutrition, University of Valladolid, Valladolid, Spain.

[6] Storr Liver Centre, Westmead Millennium Institute and Westmead Hospital, University of Sydney, NSW, Australia.

[7] Unit for the Clinical Management of Digestive Diseases. Centro para la Investigacion Biomedica en Red de Enfermedades Hepaticas y Digestivas (CIBEREHD), Virgen del Rocio-Virgen de Macarena University Hospital, University of Seville, Seville, Spain.

[8] Department of Hepatology, Sir Charles Gairdner Hospital, Nedlands Australia.

* Eduardo Vilar-Gomez and Luis Calzadilla-Bertot share first co-authorship

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List of abbreviations: NASH**,** non-alcoholic steatohepatitis; ADMs, antidiabetic medications; BMI, body mass index; NAFLD, non-alcoholic fatty liver disease; NAS, NAFLD activity score; Child Turcotte Pugh, CTP; HR, hazard ratio; T2DM, type 2 diabetes; HCC, hepatocellular carcinoma; CLD, chronic liver disease.

Correspondence:
Eduardo Vilar-Gomez, Division of Gastroenterology and Hepatology, Indiana University School of Medicine, Indianapolis, Indiana, USA. e-mail: evilar@iu.edu, Telephone: +1 (317) 278-0414.

Leon A. Adams. M503, Medical School, The University of Western Australia, Nedlands, WA 6009, Australia. e-mail: leon.adams@uwa.edu.au; Telephone: +61-8-6151-1052; Fax: +61-8-6151-1028.

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Author Contributions:
Eduardo Vilar, Luis Calzadilla-Bertot, Vincent Wong, Manuel Romero, Marlen Castellanos, Mohamed Eslam, Rocio Aller, Grace Lai-Hung Wong, Jacob George and Leon Adams were involved in the study concept and design; acquisition of data; interpretation of data; drafting of the manuscript and critical revision of the manuscript for important intellectual content. Eduardo Vilar and Luis Calzadilla-Bertot were responsible for statistical analysis. All authors approved the final draft submitted.

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Background & Aims: Factors that affect outcomes of patients with non-alcoholic steatohepatitis (NASH) related cirrhosis are unclear. We studied associations of type 2 diabetes, levels of hemoglobin A1c (HbA1c), and use antidiabetic medications with survival and liver-related events in patients with NASH and compensated cirrhosis.

Methods: We collected data from 299 patients with biopsy-proven NASH with Child-Pugh A cirrhosis from tertiary hospitals in Spain, Australia, Hong Kong, and Cuba, from April 1995 through December 2016. We obtained information on presence of type 2 diabetes, level of HbA1c, and use of antidiabetic medications. Cox proportional and competing risk models were used to estimate and compare rates of transplant-free survival, hepatic decompensation, and hepatocellular carcinoma (HCC).

Results: Two-hundred and twelve patients had type 2 diabetes at baseline and 8/87 patients developed diabetes during a median follow-up time of 5.1 y (range, 0.5–10.0 y). A lower proportion of patients with diabetes survived the entire follow-up period (38%) than of patients with no diabetes (81%) (adjusted hazard ratio [aHR], 4.23; 95% CI, 1.93–9.29). Higher proportions of patients with diabetes also had hepatic decompensation (51% vs 26% of patients with no diabetes; aHR, 2.03; 95% CI 1.005–4.11) and HCC (25% vs 7% of patients with no diabetes; aHR, 5.42; 95% CI 1.74–16.80). Averaged annual HbA1c levels over time were not associated with outcomes. Metformin use over time was associated with a significant reduction in risk of death or liver transplantation (aHR, 0.41; 95% CI, 0.26–0.45), hepatic decompensation (aHR, 0.80; 95% CI, 0.74–0.97), and HCC (aHR, 0.78; 95% CI, 0.69–0.96). Metformin significantly reduced risk of hepatic decompensation and HCC only in subjects with HbA1c levels above 7.0% (aHR, 0.97; 95% CI, 0.95–0.99 and aHR, 0.67; 95% CI, 0.43–0.94, respectively).

Conclusions: In an international cohort of patients with biopsy-proven NASH and Child-Pugh A cirrhosis, type 2 diabetes increased risk of death and liver-related outcomes, including

HCC. Patients who took metformin had higher rates of survival and lower rates of decompensation and HCC.

Key words: chemoprevention, glucose intolerance, ascites, encephalopathy, fatty liver

Non-alcoholic fatty liver disease (NAFLD) is a leading cause of cirrhosis and an increasingly common indication for liver transplantation.(1, 2) Moreover, NAFLD is responsible for an increasing number of hepatocellular carcinoma (HCC) cases with reports from the United States demonstrating a rate increase of around 9% per annum.(3, 4) Consequently, mortality rates within the US from non-alcoholic steatohepatitis (NASH) related cirrhosis and HCC have also increased significantly over the last decade.(5) Overall, the long-term clinical course of NAFLD is markedly influenced by the severity of fibrosis, whereby patients with cirrhosis are at greatest risk of developing liver-related complications (6, 7). Defining risk factors for adverse outcomes in NASH cirrhosis patients is important to identify factors that may be modifiable in order to improve outcomes. In addition, it may aid prioritization for clinical trials and for more intense monitoring.

NAFLD and T2DM frequently coexist; retrospective cohort studies including patients with biopsy-proven NAFLD have reported that T2DM increases the risk of all-cause, cardiac and liver related mortality.(8-10) Population-level data (not limited to NAFLD patients) indicates that T2DM is associated with an increased risk of HCC.(11) Moreover, T2DM has been associated with increased risk of liver-related complications (hepatic decompensation and HCC) in patients with chronic liver disease (CLD).(12, 13) However, the majority of these studies were conducted in subjects with diverse etiologies of CLD, or with limited numbers of NASH cirrhosis patients who are at most risk of liver complications. Thus,

whether T2DM increases the risk of hepatic outcomes, irrespective of severity of liver disease, remains to be elucidated.

Among patients with T2DM, the use of anti-diabetic medications (ADMs) and glycemic control may modify the risk of HCC.(14-16) Recent meta-analyses of diabetic patients have documented that metformin treatment is associated with decreased HCC risk, whereas insulin and sulphonylureas use is linked with a higher risk.(17, 18) Whether this protective effect is observed in NASH cirrhosis patients is unknown. Similar findings have been observed for overall cancers and ADMs, although it is uncertain whether this effect is independent of glycemic control.(16, 19) Thus, T2DM may represent an important modifiable risk factor through the use of ADM's or via improved glycemic control. To explore this further, we aimed to determine the influence of T2DM, hyperglycemia and ADMs on outcomes of HCC, liver decompensation and death in an international multicentre cohort of NASH patients with compensated cirrhosis.

MATERIAL AND METHODS

Study design and participants

The design and subject exclusion criteria for this international multicenter cohort study has been described previously with additional information in the Supplementary material.(6)

Overall, 458 with biopsy-proven advanced NASH (septal/bridging fibrosis or compensated cirrhosis) were followed from April 1995 until December 2016 in six tertiary hospitals from 4 countries (Spain [n=184], Australia [n=116], Hong Kong [n=82] and Cuba [n=76]). Analysis was restricted to those with compensated cirrhosis at baseline (n=299). The prospective registries and study protocol were approved by the Institutional Review Board of each participating center and all participants gave written informed consent.

Data collection

Liver histology

Liver biopsies were scored locally by expert liver pathologists using the NASH-CRN scoring system.(20) Biopsies prior to the implementation of the NASH-CRN scoring system in 2006 were re-evaluated.(6) Hepatic steatosis ≥5% with ballooning and/or lobular inflammation was required to confirm NASH diagnosis (see supplementary methods for more details).

Clinical, biochemical and demographic data

Data for demographics (age, sex, race/ethnicity), comorbidities, anthropometry, fasting serum determinations (glucose, HbA1c, insulin, lipids, liver function tests, INR, creatinine, alpha-fetoprotein), alcohol consumption and cigarette smoking within 30 days of liver biopsy were obtained.

Evaluation of glucose-related parameters and anti-diabetic medications

T2DM was defined at baseline as fasting glucose ≥126 mg/dL or HbA1c ≥6.5% or use of ADMs.(21) Data on new diagnoses of T2DM, type of ADM (metformin, sulphonylurea, insulin and others) as well as date of commencement and cessation of ADM's was recorded during follow-up from medical and pharmacy records. ADMs users were defined as patients who took drug for at least the first 6 months of follow-up. Patients were categorized into four groups: (1) Metformin users; (2) Sulfonylurea users; (3) Insulin users (4) non-ADMs users. Patients on combination medications were considered users of both drugs. (22) Use of other ADM agents (Glucagon-Like Peptide-1 Receptor agonists, sodium glucose transport protein 2 inhibitors, thiazolidinediones) was infrequent (<5% of cohort) and thus not analysed. Glycemic control during follow-up was determined according to the average annual HbA1c which was calculated for each patient by summing annual HbA1c values and dividing by the number of years of follow-up plus one, to account for the baseline value.

Follow-up and events assessment

Follow-up began on the date of biopsy, and ended on the date of the last visit, death, or transplant. A detailed medical history and physical examination along with laboratory tests were performed at each visit. Patients underwent 6 monthly ultrasound screening for HCC. Gastroscopies were performed at the discretion of the local investigator following recommended guidelines.(23) The primary outcome was all-cause death or liver transplantation. Secondary outcomes were the development of liver decompensation and HCC. Patients lost to follow-up were censored at the last date known to be alive.

Major clinical outcomes definitions

(1) Liver decompensation was defined by the: occurrence of ascites (identified clinically or by ultrasound), upper gastrointestinal bleeding secondary to portal hypertension (confirmed by endoscopy in the presence of gastroesophageal varices or hypertensive gastropathy), hepatic encephalopathy (established by clinical parameters, neuropsychological tests, or electroencephalogram).

(2) HCC diagnosed by dynamic CT scan, MRI or biopsy.

Only the index event in each category was analyzed. Outcomes occurring after liver transplantation were not considered.

Statistical analysis

The baseline characteristics were summarized in percentages (categorical variables) or mean ± SD or median and range (continuous variables). Categorical variables were compared using χ^2 test. Continuous variables were compared using the t test for normally distributed variables and the nonparametric Wilcoxon rank-sum test for measures not normally distributed.

The primary analysis was to explore the influence of diabetes, ADM's and HbA_{1c} values on death/transplant and hepatic outcomes (see supplementary methods).

The cumulative probability of death or transplant based on T2DM status, HbA1c cut-offs and ADMs was analyzed by the Cox proportional method. Cumulative incidences of secondary outcomes (first event of hepatic decompensation and HCC), were calculated in the presence of competing risks events.(24) Annualized incidence rates and 95% confidence intervals for outcomes among diabetic and non-diabetic patients were reported. Missing values were imputed by applying the multiple imputations method (see supplementary methods). All confidence intervals, significance tests, and resulting *P* values were two-sided, with an alpha level of 0.05. Statistical analyses were performed using STATA software, release 15.

RESULTS

Of 512 patients within the cohort, 299 patients had NASH-related cirrhosis and fulfilled the inclusion criteria (**Figure 1**). The overall follow-up was a median of 5.1 years (range 0.5-10 years). Compared with non-diabetic patients (n=87), diabetics (n=212) at baseline were older, had more hypertension, gastroesophageal varices and worse renal function (**Table 1).** Of the 87 patients without diabetes at baseline, eight (9%) developed type 2 diabetes during follow-up, a mean of 5.5 (± 1.3) years following liver biopsy. The majority of diabetic patients (79%) were on ADMs including metformin (n=111), sulfonylureas (n=61) and insulin (n=87). Less frequent ADMs were: pioglitazone/rosiglitazone (3%), DPP-4 (4%) or SGLT2i (3%). Insulin users displayed higher HbA1c and creatinine values as compared with metformin and sulphonylureas users **(supplementary Table 1).**

Type 2 diabetes mellitus: Impact on transplant-free survival and hepatic outcomes.

Overall survival and transplant

A total of 70 patients died (n=33) or underwent liver transplantation (n=37). Most deaths were liver-related (29 of 33, 87%). Causes of death and occurrence of major clinical events among diabetic and non-diabetic patients are summarized in **supplementary Table 2.**

Ten-year survival without transplantation was significantly lower in diabetic patients (38%, 95% CI:31-45) compared with non-diabetics (81%, 95% CI:75-88). Annualized mortality or transplant rates were 4.9 and 3.0 /100 person-years, in diabetic and non-diabetic patients respectively, (Cox-adjusted $P<0.01$, **Figure 2A, Table 2**). Other predictors of overall mortality/transplant rates on univariate analysis are reported in **Supplementary Table 3**. Multivariable Cox-adjusted analysis revealed T2DM increased the risk of all-cause mortality or transplant 4.59 (95% CI:2.23-9.43) times as compared with no T2DM. This remained significant when analysing T2DM as a time-dependent variable (adj. HR 4.23, 95% CI:1.93-9.29, p<0.001).

Hepatocellular carcinoma

Thirty-nine patients developed HCC; Nine underwent transplantation and seven died due to HCC. At 10 years, patients with diabetes were more likely to develop HCC (25%, 95% CI:18-30) than those without diabetes (7%, 95% CI:3-13), $P<0.01$. The annualized rates (/100 person-years) for HCC were 3.1 and 1.2 in diabetic and non-diabetic patients, respectively (**Figure 2B, Table 2**). On multivariate analysis, a 4.2-fold (95% CI:1.2-14.2) increased risk of HCC was observed among diabetics versus non-diabetics. T2DM remained a significant predictor of HCC when analysed as a time-dependent variable (adj.sHR 5.42, 95% CI:1.74-16.80, p=0.003).

Hepatic decompensation

Ascites (60/83) and variceal bleeding (18/83) were the most common initial events of decompensation. The 10-year adjusted cumulative incidence of hepatic decompensation was higher in patients with T2DM (51%, 95% CI:44-59) than those without T2DM (26%, 95%

CI:17-33), P<0.01, corresponding to annual rates of 6.6 and 4.2, respectively (**Figure 2C, Table 2**). T2DM increased the risk of hepatic decompensation 2.46 times (95% CI:1.35-4.46) compared with no T2DM. When analysed as a time-dependent co-variate, T2DM remained significantly predictive of decompensation (adj.sHR 2.03, 95% CI:1.005-4.11, p=0.048).

Anti-diabetic medications: Impact on transplant-free survival and hepatic outcomes

An overview of antidiabetic medications duration, cessation and new users during follow up is described in Supplementary material. Use of metformin at baseline, compared to non-use, was associated with a higher cumulative probability of transplant-free survival (78%, 95% CI:71-85 vs. 44%, 95% CI:37-52 *P*<0.01), and lower cumulative incidence of hepatic decompensation (45%, 95% CI:37-52 vs. 60%, 95% CI:55-68, *P*=0.047), **Figure 3A-B**. HCC rates were not significantly different in baseline metformin users (10%, 95% CI:6-17) vs. non-users (14%, 95% CI:8-22), **Figure 3C**. At multivariable analysis (**Table 3**), metformin (as a time dependent variable) was independently associated with reduced risk of all-cause mortality (HR: 0.41, 95% CI:0.26-0.45, *P*<0.001), hepatic decompensation (sHR: 0.80, 95% CI:0.74-0.97, P=0.005), and HCC (sHR 0.78, 95% CI:0.69-0.96), P=0.047**)**. Adjustment for statin use or HOMA-IR did not change the association between metformin and outcomes (data not shown).

The benefits of metformin use on transplant-free survival were observed in patients with an HbA1c ≤7% (n=178) and >7% (n=121) (adj.HR: 0.93, 95% CI:0.89-0.98 and adj.HR 0.87, 95% CI:0.84-0.90 respectively), however the association with hepatic decompensation and HCC was only seen in patients with HbA1c of >7% (adj.sHR 0.97, 95% CI:0.95-0.99 and adj.sHR 0.67, 95% CI:0.45-0.94 respectively), outlined in **Supplemental Table 4.**

HbA_{1c} and HOMA-IR levels: Influence on transplant-free survival and hepatic outcomes.

Overall, average annual HbA1c values were not associated with transplant free survival [HR:1.09 (95% CI:0.92-1.29), P=0.30], hepatic decompensation [sHR:1.07 (95% CI:0.93-1.24), P=0.32] or development of HCC [sHR:1.11 (0.89-1.37), P=0.34]. Whilst the risk of overall mortality or transplant tended to increase per quartile of average annual HbA1c values, this did not reach statistical significance (**Supplementary Table 5**, **Supplementary Figure 1A-C).** Among diabetic patients, HOMA-IR at baseline was not associated with outcomes (**Supplementary Table 6**). Among diabetics not taking insulin at baseline, HOMA-IR was associated with an increased risk of mortality/transplant on univariate analysis, however this did not remain significant following adjustment for potential confounders (HR 0.99, 95% CI 0.94-1.04, p=0.9).

DISCUSSION

In this multicenter, international cohort study of patients with biopsy-proven NASH cirrhosis, diabetic patients were at significantly higher risk of adverse outcomes compared to non-diabetics; one quarter of NASH-cirrhosis patients with T2DM died or underwent liver transplantation during a median of five years follow-up, with these patients having more than four-fold increased risk compared to those without diabetes. Similarly, T2DM was associated with a higher risk of hepatic decompensation and HCC even following adjustment for major confounders including use of glucose-lowering medications. Significantly, metformin use had a protective association for overall mortality and transplant, hepatic decompensation and HCC.

Although T2DM has been associated with detrimental liver outcomes among patients with the full-histological spectrum of NAFLD, its impact on survival and liver-related events

in cirrhotic patients has been unclear.(10, 25) Population-based studies have demonstrated T2DM to be associated with higher standardized mortality ratios for liver related death, although the absolute risk of death in patients with T2DM in these cohorts remained low.(10, 26, 27) Our data clearly demonstrates that in the presence of compensated cirrhosis, T2DM identifies an at-risk group at high probability of developing liver related morbidity and mortality over the medium term, thereby indicating a need for heightened surveillance and prioritization for effective therapy. There was an unexpected low rate of death from cardiovascular disease in T2DM patients with NASH cirrhosis, which may reflect the reduction in blood pressure, body weight and cholesterol which occurs with progressive fibrosis, or due to competing risks from liver disease limiting opportunity for cardiovascular disease to manifest.

Growing evidence has linked T2DM and HCC, independently of recognized confounding factors such as obesity, age, sex, patterns of alcohol consumption, cirrhosis and chronic viral hepatitis.(4, 28, 29) Nonetheless, a single-center cohort study and multicentre case-control study both failed to demonstrate a link between HCC and T2D in NASH cirrhosis patients.(30, 31) In contrast, our data demonstrated T2DM to be robustly associated with HCC risk, irrespective of well-known confounders, likely due to the larger sample size and follow-up.

The use of metformin was associated with a 59%, 20% and 22% reduction in risk of all-cause mortality or transplant, hepatic decompensation and HCC respectively. Notably, no patient developed lactic acidosis or serious complications from metformin use, in line with other reports.(32) However, the safety and efficacy of metformin in decompensated cirrhosis is not clear and caution should be applied using it. Clinical trials have been conflicting regarding the impact of metformin on liver histology in non-cirrhotic NASH patients, and a lack of adequately powered randomized controlled trials has prevented recommendation for

its use in patients with NASH.(33, 34) Interestingly, the continued use of metformin after cirrhosis diagnosis was associated with improved survival in a single center study, particularly among NASH cirrhosis patients, although it is unclear whether this was due to reduced rates of liver decompensation, HCC or extra-hepatic disease.(32) The protective effect of metformin in a NASH population was also recently noted in a retrospective study based on 191 biopsy proven NASH (86% cirrhosis) patients where metformin users had a lower risk of all-cause death and liver transplant and HCC compared to non-users.(35) Given the significant reduction in mortality associated with metformin use in our cohort, our data provides rationale to examine the impact of metformin in a prospective randomized clinical trial on NASH cirrhosis outcomes. We lacked data on newer ADM's such as thiozoladinediones and glucagon-like peptide-1 receptor agonists. These agents came into routine clinical use relatively late in the follow-up period and it is likely the study would have been under-powered to detect a significant association with outcomes.

In the context of HCC, results from several clinical studies have indicated that type 2 diabetic patients treated with metformin might have a lower risk of any cancer (36) and this protective effect appears to be particularly important for gastrointestinal- malignancies including HCC.(17, 37) We did not find a protective association between metformin and HCC, though there was a non-significant trend, raising the possibility that our study could be underpowered to detect an effect.

Our study has a number of strengths, being a multicenter, international and multiethnic study consisting of a large cohort of patients with histologically-confirmed cirrhosis followed for a long period with very few patients lost to follow-up. To our knowledge, this is the biggest cohort of biopsy proven NASH patients with compensated cirrhosis in which the influence of diabetes, its medications and baseline glucose levels on survival and liver-related outcomes has been investigated. Our results also need to be interpreted with caution because

of study limitations. Firstly, it is a retrospective analysis with risk of bias, although this has been minimized by standardized baseline data collection at each unit, minimal loss to follow-up and the examination of hard outcomes such as mortality. Second, although HbA1c is an accurate diagnostic test for T2DM in Childs A cirrhosis (area under the curve 0.85), we cannot exclude that anemia or elevated red cell turnover may have impacted on HbA1c levels and its relationship on outcomes over time.(38) In addition, it is possible that HbA1c progressively underestimated glucose control during long-term follow-up in patients who developed worsening cirrhosis, such that any association with worsening glycemic control and outcomes was missed.

In conclusion, in this cohort study, T2DM was associated with an increased risk of death, hepatic decompensation and HCC. These associations suggest the need for close surveillance for these high-risk patients in order to reduce death and complications. Metformin may lower the risk of death and liver-related events and warrants further well-designed studies to clarify the beneficial or harmful effects of ADMs on outcomes among NASH cirrhosis patients with diabetes.

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FIGURES

Figure 1. Flow diagram of patients in the study

Figure 2. Adjusted cumulative probability of outcomes according to presence of Type 2 diabetes.

(A) Transplant-free survival

(B) First occurrence of hepatic decompensation

(C) First occurrence of hepatocellular carcinoma

* Slopes and *P* values represent adjusted predictions by center, calendar year of patients' recruitment, age, sex, race/ethnicity, BMI, smoking status, alcohol consumption, CTP score, esophageal varices, HbA1c levels and glucose-lowering medications at baseline.

Fig. 3. Impact of metformin use on major outcomes. Analyses based on all-users versus non-users.

(A) Cox model-adjusted *transplant-free survival rates.

(B) Competing risk-adjusted *hepatic decompensation rates.

(C) Competing risk-adjusted *hepatocellular carcinoma rates.

* Slopes represent adjusted predictions by center, calendar year of patients' recruitment, age, sex, race/ethnicity, BMI, smoking status, alcohol consumption, CTP score, esophageal varices, HbA1c levels and glucose-lowering medications at baseline.

Hazard ratio's are reported using metformin as a time-dependent co-variate.

Table 1. Baseline characteristics.

Variable	No type 2 diabetes n=87	Type 2 diabetes n=212	*P* value
Age (y)	52.5 ± 13.2	58.9 ± 10.3	<0.01
Male, n (%)	38 (44)	101 (48)	0.53
Race/ethnicity			0.02
Hispanic white	57 (66)	123 (58)	
Non-Hispanic white	20 (23)	47 (22)	
Asian	7 (8)	41 (19)	
Black	3 (3)	1 (1)	
Current smoker	18 (21)	34 (16)	0.61
BMI (kg/m^2)	33.1 ± 8.8	31.8 ± 6.1	0.67
Waist (cm)	105.5 ± 15.3	106.1 ± 16.1	0.95
Length of follow-up	5.6 (3.1-8.8)	5.0 (2.5-8.3)	0.10
MELD score	8.0 ± 2.5	8.4 ± 3.1	0.35
Child-Pugh score, n (%)			
Class A6	21 (24)	56 (26)	0.68
Gastroesophageal varices, n (%)	10 (11)	75 (35)	<0.01
History of hypertension, n (%)	43 (49)	141 (67)	<0.01
Anti-diabetic medications, n (%)	0 (0)	168 (79)	-
Non-users, n (%)	0 (0)	44 (21)	-
Metformin users, n (%)	0 (0)	111 (52)	-
Sulfonylureas users, n (%)	0 (0)	62 (29)	-
Insulin users, n (%)	0 (0)	87 (41)	-
DDP-4 inhibitors, n (%)	0 (0)	8 (4)	-
SGLT2 inhibitors, n (%)	0 (0)	6 (3)	-
Glitazones, n (%)	0 (0)	7 (3)	-
ALT (U/L)	79 ± 55	56 ± 37	0.35
AST (U/L)	83 ± 59	52 ± 31	0.10
AST/ALT ratio	1.18 ± 0.57	1.07 ± 0.39	0.47
γ-Glutamyl transferase (U/L)	116 ± 98	155 ± 135	0.02
Fasting glucose (mg/dl)	92.8 ± 12.1	168.1 ± 62.8	<0.01

HbA1c (%)	5.39 ± 0.51	7.82 ± 2.07	<0.01
Fasting insulin (mIU/L)	16.2 ± 8.9	24.8 ± 13.2	<0.01
HOMA-IR	3.78 ± 2.3	11.26 ± 9.01	<0.01
Total cholesterol (mg/dl)	175.4 ± 47.3	176.4 ± 54.8	0.95
HDL cholesterol (mg/dl)	45.9 ± 13.2	43.1 ± 10.6	0.27
LDL cholesterol (mg/dl)	99.3 ± 39.9	104.9 ± 47.9	0.59
Triglycerides (mg/dl)	149.4 ± 69.8	163.9 ± 79.5	0.14
Statin therapy, n (%)	14 (16)	79 (37)	<0.01
Total bilirubin (mg/dl)	1.07 ± 0.84	0.91 ± 0.67	0.07
Albumin (g/dl)	4.14 ± 0.41	4.16 ± 0.40	0.53
INR	1.13 ± 0.33	1.09 ± 0.16	0.80
Platelets (x 10^9/L)	172 ± 65	165 ± 64	0.31
α-fetoprotein (ng/ml)	3.6 ± 1.8	3.7 ± 1.8	0.92
Creatinine (mg/dl)	0.80 ± 0.19	0.93 ± 0.46	<0.01
eGFR, mL/min/1.73m^2	93.9 ± 17.7	81.7 ± 24.7	<0.01
eGFR <60 mL/min/1.73m^2	3 (3)	42 (20)	<0.01
History of vascular disease, n (%)	9 (7)	19 (9)	0.55
History of malignancy, n (%)	1 (1)	7 (3)	0.30
Aspirin therapy, n (%)	4 (5)	10 (6)	0.96
Biopsy length (mm)	18.9 ± 4.9	18.8 ± 5.01	0.83
Portal tracts (n)	9.36 ± 2.5	9.58 ± 2.4	0.18
NAS	3.82 ± 1.83	4.11 ± 1.87	0.21
NAS ≥ 4, n (%) *	46 (53)	134 (63)	0.10
Individual component of NAS			
Steatosis	1.68 ± 0.81	1.63 ± 0.87	0.69
Lobular inflammation	1.17 ± 0.36	1.37 ± 0.81	0.07
Ballooning	0.97 ± 0.76	1.11 ± 0.74	0.17
Country, n (%)			0.09
Spain§	36 (41)	82 (39)	
Australia	20 (23)	48 (23)	
Hong-Kong	7 (8)	40 (18)	
Cuba	24 (28)	42 (20)	

Abbreviations: BMI, body mass index; MELD, Model for End-Stage Liver Disease; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HbA1c, glycated hemoglobin; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; INR, international normalized ratio; eGFR, estimated glomerular filtration rate; NAS, NAFLD activity score.

Quantitative data expressed as mean ± SD.

Length of follow variable expressed as median and range

For all laboratory measures and for continuous demographics: T test or Wilcoxon rank-sum test.

Proportions: percentage, Chi-Square test.

The eGFR was computed by EPI-CKD formula.

* NAS indicates NAFLD activity score. It was defined as the unweighted sum of the scores for steatosis (0-3), lobular inflammation (0-3), and ballooning (0-2); thus, ranging from 0 to 8.

Table 2. Annualized incidence rates of clinical outcomes in NASH cirrhosis patients according to baseline diabetes status.

Variable	No diabetes n=87			Diabetes n=212		
	No.	Rates	95% CI	No.	Rates	95% CI
All deaths or transplantations	15	3.00	1.81-4.98	55	4.89	3.76-6.37
Hepatic decompensation	19	4.19	2.67-6.57	64	6.63	5.19-8.48
Development of HCC	6	1.21	0.54-2.70	33	3.11	2.21-4.37
Total major vascular events*	1	0.28	0.02-1.42	5	0.45	0.18-1.07
Total non-hepatic malignancies	6	1.25	0.56-2.77	7	0.63	0.30-1.33

* Major vascular events included cardiovascular, cerebrovascular, and arterial peripheral diseases.

Recurrence of clinical events and skin cancers were not included.

Table 3. Risk of mortality/transplant, hepatic decompensation and HCC based on the use of anti-diabetic medications overtime.

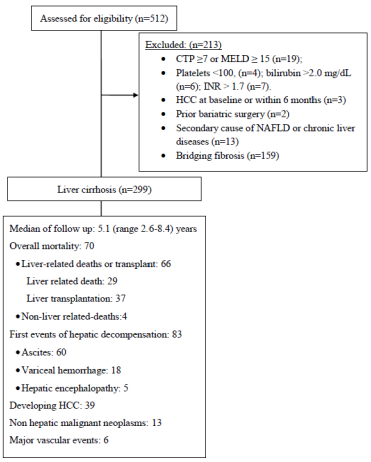
ADMs	Hazard* or subhazard† ratio (95% CI) Unadjusted	P value	Hazard* or subhazard† ratio (95% CI) Adjusted‡	P value
Mortality or transplant				
Metformin	0.32 (0.24-0.41)	<0.001	0.41 (0.26-0.45)	<0.001
Insulin	1.31 (0.74-2.29)	0.34	0.85 (0.41-1.78)	0.66
Sulphonylurea	0.76 (0.41-1.41)	0.39	1.26 (0.65-2.46)	0.48
Hepatic decompensation				
Metformin	0.78 (0.68-0.90)	0.001	0.80 (0.74-0.97)	0.005
Insulin	1.72 (1.01-2.92)	0.04	2.30 (1.19-4.44)	0.012
Sulphonylurea	0.62 (0.36-1.05)	0.08	0.83 (0.49-1.43)	0.519
Hepatocellular carcinoma				
Metformin	0.27 (0.64-1.68)	0.16	0.78 (0.69-0.96)	0.047
Insulin	1.92 (0.91-4.05)	0.08	1.84 (0.65-5.19)	0.244
Sulphonylurea	0.60 (0.26-1.42)	0.25	0.96 (0.36-2.61)	0.947

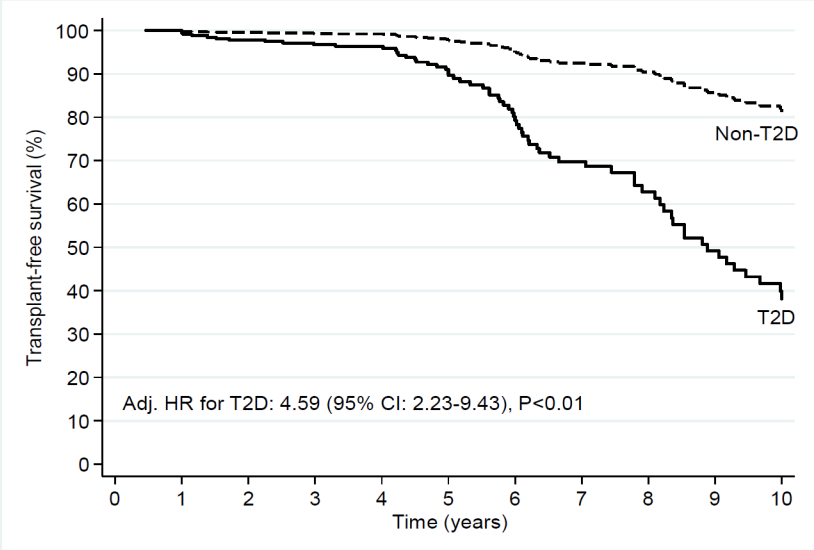
***** Cox proportional regression hazard models.

† Competing risk regression models.

‡ Adjustments by center, calendar year of patients' recruitment, age, sex, race/ethnicity, BMI, smoking status, alcohol consumption, CTP score, esophageal varices, annual average HbA1c levels. ADM's were analysed as time-dependent co-variates.

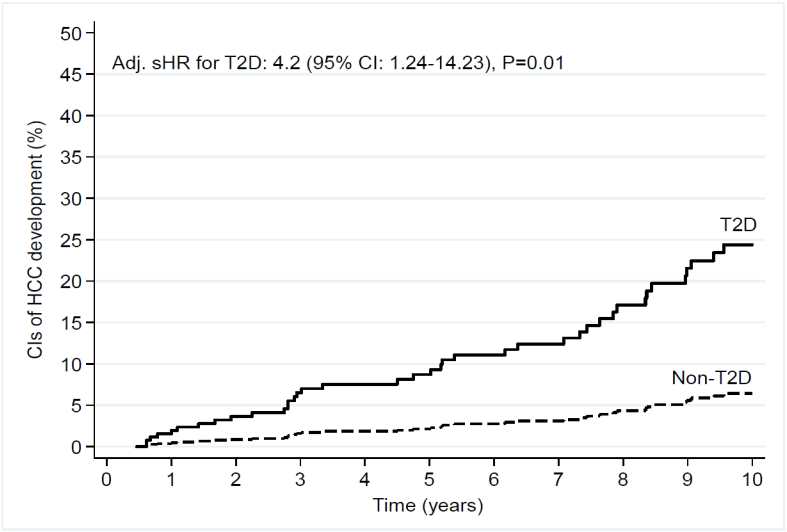
Abbreviations: ADMs, antidiabetic medications; CI, confidence interval.


Assessed for eligibility (n=512)
Excluded: (n=213)
CTP ≥7 or MELD ≥ 15 (n=19);
Platelets <100, (n=4); bilirubin >2.0 mg/dL (n=6); INR > 1.7 (n=7).
HCC at baseline or within 6 months (n=3)
Prior bariatric surgery (n=2)
Secondary cause of NAFLD or chronic liver diseases (n=13)
Bridging fibrosis (n=159)
Liver cirrhosis (n=299)
Median of follow up: 5.1 (range 2.6-8.4) years
Overall mortality: 70
Liver-related deaths or transplant: 66
Liver related death: 29
Liver transplantation: 37
Non-liver related-deaths:4
First events of hepatic decompensation: 83
Ascites: 60
Variceal hemorrhage: 18
Hepatic encephalopathy: 5
Developing HCC: 39
Non hepatic malignant neoplasms: 13
Major vascular events: 6



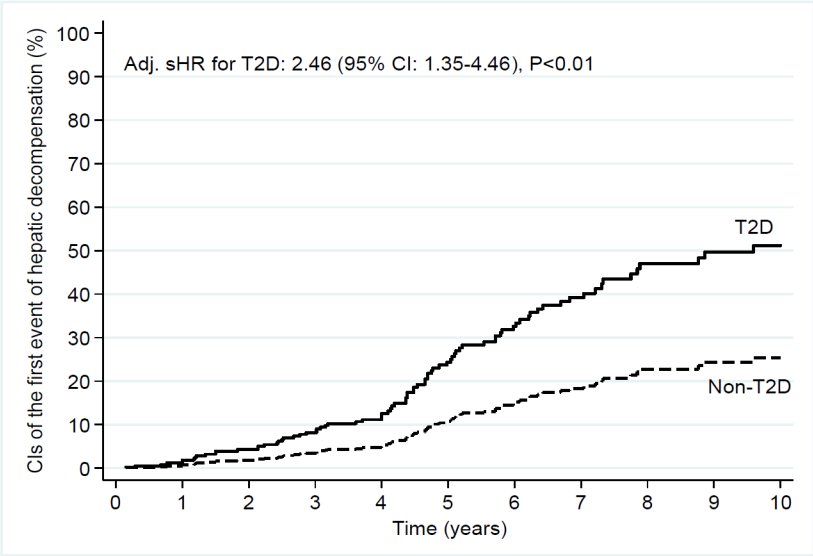
No. at risk

T2D	212	200	175	143	125	106	86	70	56	46	34
No T2D	87	86	74	67	57	50	40	31	25	21	14

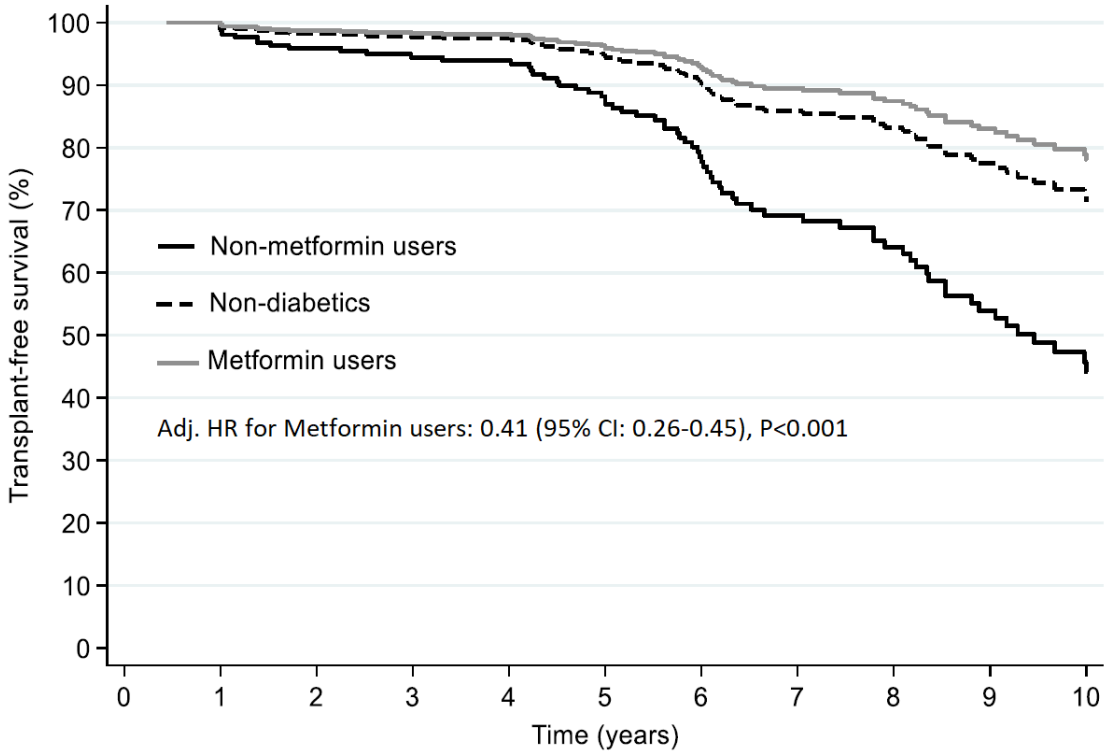


No. at risk

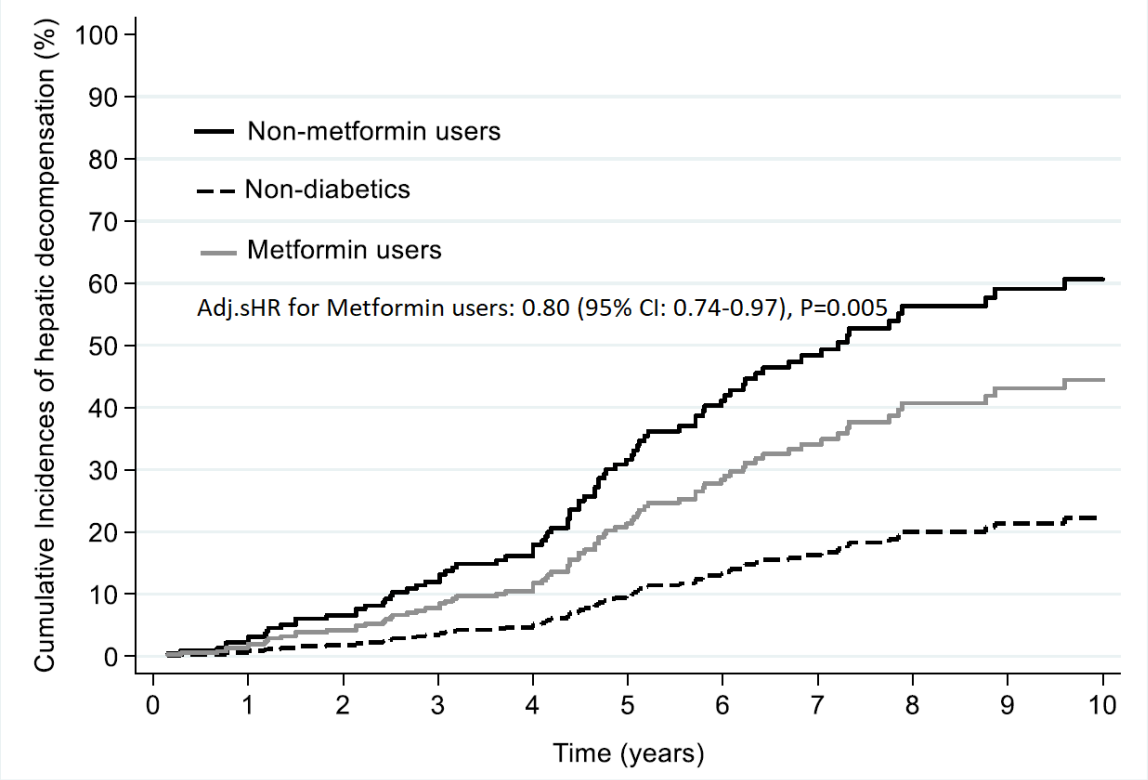
T2D	212	198	172	138	118	98	76	62	47	37	28
No T2D	87	86	74	66	57	49	40	31	24	19	12



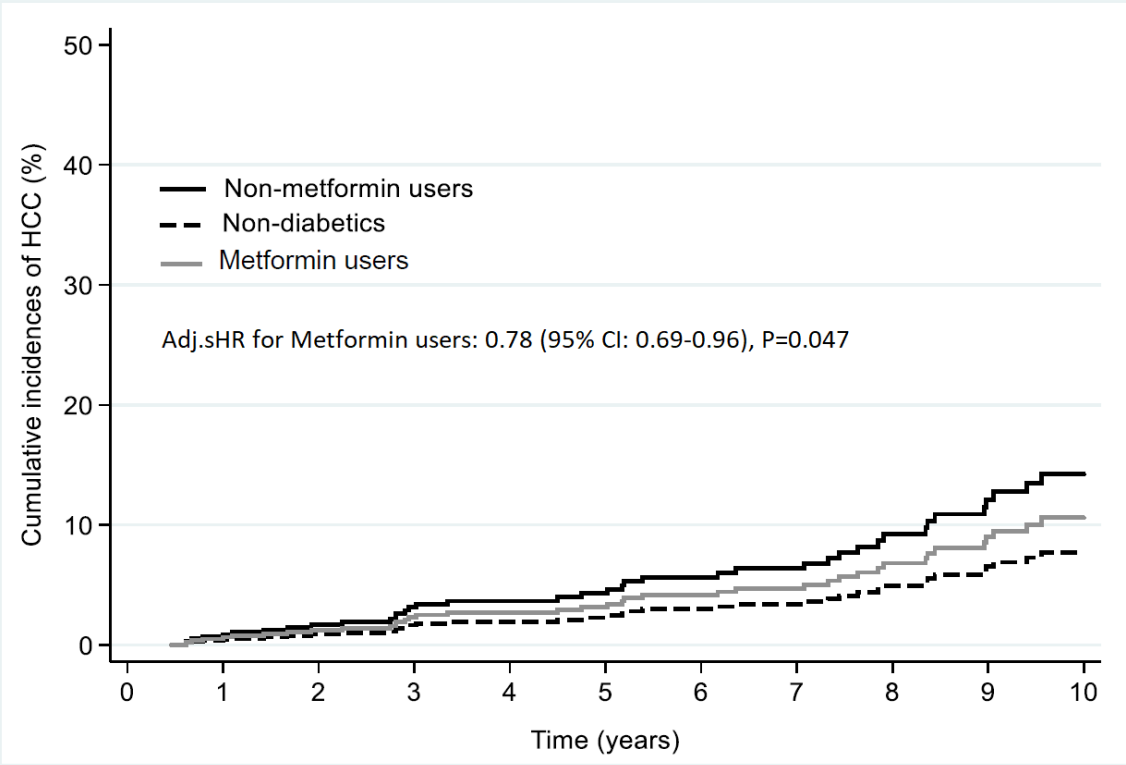
No. at risk											
T2D	212	197	167	132	108	77	62	50	34	30	21
No T2D	87	84	73	64	53	44	31	22	19	17	11



No. at risk											
T2D - Met	111	108	94	74	61	55	50	44	33	27	17
T2D no Met	101	92	81	69	64	51	36	26	23	19	17
No T2D	87	86	74	67	57	50	40	31	25	21	14



No. at risk											
T2D - Met	111	106	92	69	51	42	34	31	22	19	10
T2D no Met	101	91	75	63	57	35	28	19	12	11	11
No T2D	87	84	73	64	53	44	31	22	19	17	11



No. at risk											
T2D - Met	111	107	93	69	54	48	42	36	27	22	15
T2D no Met	101	91	79	69	64	50	34	26	20	15	13
No T2D	87	86	74	66	57	49	40	31	24	19	12

What you need to know:

Background: Little is known about the effects of type 2 diabetes and diabetic medications on long-term outcomes of patients with non-alcoholic steatohepatitis (NASH)-related cirrhosis.

Findings Type 2 diabetes significantly increased risk of death and liver transplantation, hepatic decompensation, and hepatocellular carcinoma in patients with NASH-related cirrhosis. Use of metformin was associated with a reduced risk of death or liver transplant, hepatic decompensation, and hepatocellular carcinoma.

Implications for patient care: Patients with NASH and diabetes are at increased risk of adverse outcomes, compared to patients with NASH without diabetes. Metformin use might reduce the risk of liver-related morbidity and mortality in this group.